Original Article

Frequency of C4d Positivity in Membranous and other Glomerulonephritis in Renal Biopsy Specimens in a Tertiary Care Hospital

Maria Shafique, Uzma Bukhari, Suresh Kumar, Muhammad Raza, Asma Bukhari

ABSTRACT

OBJECTIVE: To determine the frequency of C4d positivity in Membranous and other Glomerulonephritis in renal biopsy specimens.

METHODOLOGY: This cross-sectional study was carried out in the Pathology Department, Ziauddin Medical University and Hospital, Karachi, from December 2018 to April 2019. Non-probability consecutive sampling technique was used.. A sample size of 91 patients was calculated and selected by open EPI software with a 95% confidence interval. All patients of either gender with a clinical history of Glomerulonephritis were included. The diagnosis was made on sections stained with hematoxylin and eosin (H&E) along with special stains. Immunohistochemistry was performed on selected biopsies with glomerular basement thickness using the peroxidase-anti peroxidase technique for C4d. Patient data were compiled and analyzed through a statistical package for the Social Sciences (SPSS) Version 20.0. P -value ≤0.05 was considered significant.

RESULTS: A total of 91 cases of the renal biopsy were evaluated. There were 51.6% (47/91) were male, and 48.4% (44/91) were female patients. There were 49 (53.8%) cases reported as Focal segmental glomerulosclerosis, 35(38.5%) were diagnosed as Membranous Glomerulonephritis, and 07 (7.7%) cases were reported as Mesangiocapillary Glomerulonephritis.

The frequency distribution of C4d staining results in Membranous Glomerulonephritis was 100% (35/91), Mesangiocapillary glomerulonephritis 100% (7/91), and Focal segmental glomerulosclerosis was 0% (0/91).

CONCLUSION: C4d positivity on immunohistochemistry (IHC) can be an important marker for diagnosing membranous nephropathy.

KEY WORDS: Glomerulonephritis, Immunofluorescence, immunohistochemistry C4d Positivity.

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INTRODUCTION

Glomerular diseases are a public health concern throughout the world. According to the World Health Statistics and Sustainable Development Goals (SDG) project, diseases in the renal and urinary system account for a great deal of the global burden of diseases, with an estimated eight hundred and fifty thousand annual deaths and over fifteen million disability-adjusted life years [1].

Membranous Glomerulonephritis is the general cause of the adult nephrotic syndrome. Approximately 25% of renal biopsies were performed for this syndrome. The diagnosis is established with the patient's presenting signs and symptoms and findings on H&E staining showing thickening of the glomerular capillary wall. However, cases of early membranous may not show apparent thickening and spikes[2].

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Thus, immunofluorescence (IF) examination is necessary, showing immunoglobulin G (IgG) and or C3 with granular staining. In biopsies, which have subtle spikes or / no spikes on methenamine silver stain or no glomeruli on Immunofluorescence, C4d positivity on immunohistochemistry (IHC) played a valuable tool in established diagnosis as membranous nephropathy in multiple international studies[3,4].

The vital role of immunohistochemistry (IHC) in appreciating C4d immunological consequences in renal pathology is considerable. Its staining is not expensive and can be efficiently performed in laboratories with uncomplicated results analysis. Multiple authors have compared IF and IHC in renal diseases by giving specific attention to the specificity and sensitivity[5,6]. It has been concluded that the pattern and intensity analysis between IF and IHC are commensurate.

There are limited compositions present in the use of C4d for Glomerulonephritis. Furthermore, no local study on its results in membranous and other

Glomerulonephritis has been done. Therefore, the current research will highlight the role of C4d in membranous and other Glomerulonephritis.

METHODOLOGY

Our cross-sectional study was conducted in the Department of Pathology, Dr Ziauddin Medical University and Hospital, Karachi. Sample collection was carried out from December 2018 to April 2019. Non-probability consecutive sampling technique was used for the study. A sample size of 91 patients with Glomerulonephritis was calculated and selected by openEPI software with a 95% confidence interval and margin of error of 4.5%, with a prevalence of C4d positivity of 95%.

All patients of either gender with a clinical history of Glomerulonephritis with presenting symptoms of nephrotic or nephritic syndrome, e.g., hematuria, proteinuria, hypertension, edema, were included in the study. Patients with no clinical history of glomerulonephritis and transplant biopsies were omitted. Informed consent from the patient was taken. The diagnosis was made on formalin-fixed and paraffin-embedded tissue sections stained with hematoxylin and eosin (H&E) and special stains, including periodic acid Schiff (PAS) trichrome methenamine silver. Biopsies with an optimal number of glomeruli (10-11) counted on H&E stains were noted. Immunohistochemistry (IHC) was performed on selected biopsies with glomerular basement membrane thickness using the peroxidase-anti peroxidase technique for C4d.

Patient data were compiled and analyzed through a statistical package for the Social Sciences (SPSS) Version 20.0. Mean±SD were calculated for quantitative variable, i.e., a number of glomeruli. The stratification was done on the number of glomeruli to see the effect of these modifiers on outcomes. After stratification, the Chi-square test was used to assess significance, and P-value ≤0.05 was considered significant.

RESULTS

A total of 91 cases of renal biopsy were evaluated. There were 51.6% (47/91) were male, and 48.4% (44/91) were female patients. The overall mean age of patients was 30.71±14.99 years. There were 49 (53.8%) cases reported as Focal segmental glomerulosclerosis (Figure I), 35(38.5%) cases were diagnosed as Membranous Glomerulonephritis (Figure II, III), and 07 (7.7%) cases were reported as Mesangiocapillary Glomerulonephritis. Out of 35 cases of Membranous Glomerulonephritis, seven were reported as pre-spike Membranous Glomerulonephritis on H&E stain. Diffuse GBM thickening with spikes has been seen in 32 cases on silver stains.

Descriptive statistics of focal segmental glomerulosclerosis, membranous Glomerulonephritis and mesangiocapillary Glomerulonephritis according to gender, age group and glomeruli number are presented in the Tables I, II and III, respectively.

The frequency distribution of C4d staining results according to membranous Glomerulonephritis was 100% (35/91) (Figure IV), mesangiocapillary glomerulonephritis 100% (7/91), and focal segmental glomerulosclerosis 0% (0/91). The mean number of glomeruli positive on C4d staining was 19.82±11.31 Frequency of C4d staining results according to gender, age group and glomeruli number is presented in Table IV.

FIGURE I: PAS STAIN SHOWS FOCAL SEGMENTAL GLOMERULOSCLEROSIS

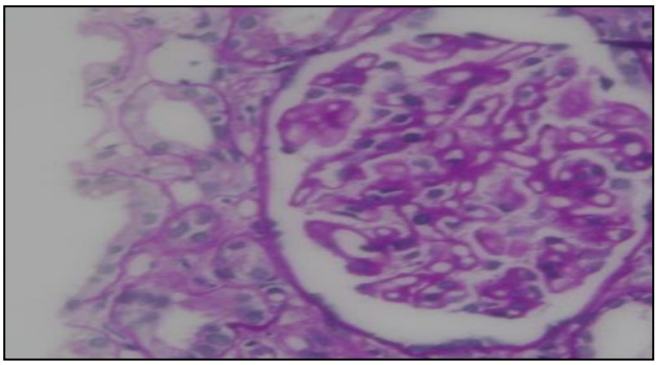


FIGURE II: SILVER STAIN SHOWS MEMBRANOUS GLOMERULONEPHRITIS

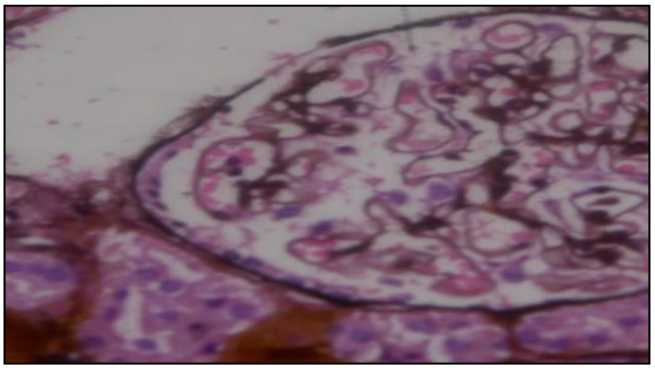


FIGURE III: IMMUNOFLUORESCENCE SHOWS POSITIVE IGG IN MEMBRANOUS GLOMERULONEPHRITIS

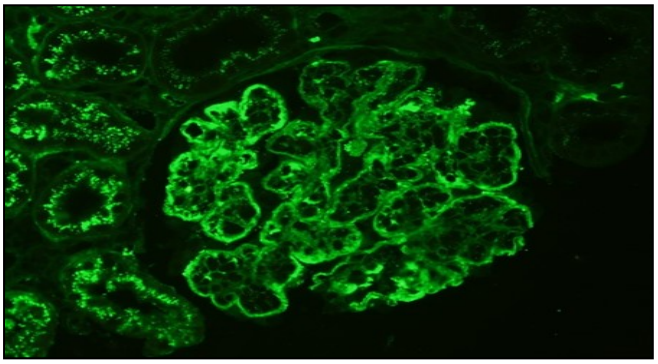


TABLE I: FREQUENCY OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS ACCORDING TO GENDER, AGE GROUP AND GLOMERULI NUMBER (n=91)

		Total	Focal Segmental Glomerulosclerosis		P-Value
			Yes (n=49)	No (n=42)	
Gender	Male	47	29(61.7)	18(38.3)	0.120
	Female	44	20(45.5)	24(54.5)	
Age Group	≤18 years	24	13(54.2)	11(45.8)	0.671
	>18 years	47	36(53.7)	31(46.3)	
Glomeruli number	≤25	44	22(50)	22(50)	0.476
	>25	47	27(57.4)	20(42.6)	

TABLE II: FREQUENCY OF MEMBRANOUS GLOMERULONEPHRITIS ACCORDING TO GENDER, AGE GROUP AND GLOMERULI NUMBER (n=91)

		Total	Membranous Glomerulonephritis		P-Value
			Yes (n=35)	No (n=56)	
Gender	Male	47	15(31.9)	32(68.1)	0.185
	Female	44	20(45.5)	24(54.5)	
Age Group	≤18 years	24	9(37.5)	15(62.5)	0.910
	>18 years	67	26(38.8)	41(61.2)	
Glomeruli number	≤25	44	21(47.7)	23(52.3)	0.079
	>25	47	14(29.8)	33(70.2)	

TABLE III: FREQUENCY OF MESANGIOCAPILLARY GLOMERULONEPHRITIS ACCORDING TO GENDER, AGE GROUP AND GLOMERULI NUMBER (n=91)

		Total	Mesangiocapillary Glomerulonephritis		P-Value
			Yes (n=7)	No (n=84)	
Gender	Male	47	3(6.4)	44(93.6)	0.628
	Female	44	4(9.1)	40(90.9)	
Age Group	≤18 years	24	2(8.3)	22(91.7)	0.891
	>18 years	67	5(7.5)	62(92.5)	
Glomeruli number	≤25	44	1(2.3)	43(97.7)	0.060
	>25	47	6(12.8)	41(87.2)	

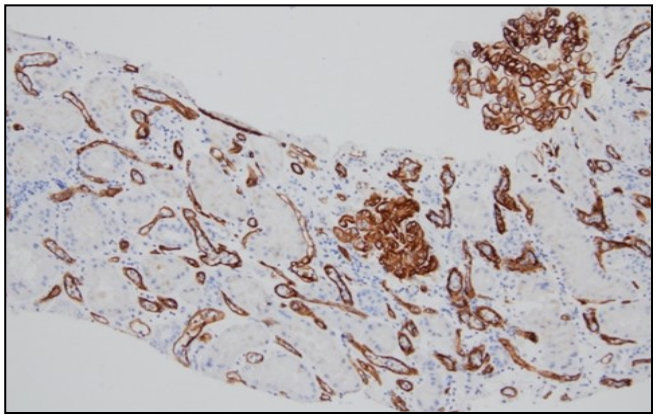
DISCUSSION

The glomerular disease rate is variable in various populations with unique genetic and demographic characteristics[7]. Chronic kidney disease is 12.5% common in Karachi. While detailing the incidence of CKD in Pakistan as 64%[8]. Furthermore, the findings from a study conducted in Karachi suggest that the glomerular filtration rate was reduced to some degree in nearly a quarter of all screened individuals. In most renal dysfunction cases (81.06%), the underlying pathology is glomerular dysfunction/disease[9].

TABLE IV: FREQUENCY OF C4d STAINING RESULTS ACCORDING TO GENDER, AGE GROUP AND GLOMERULI NUMBER (n=91)

		Total	C4d Staining Result		P-Value
			Yes (n=35)	No (n=56)	
Gender	Male	47	15(31.9)	32(68.1)	0.185
	Female	44	20(45.5)	24(54.5)	
Age Group	≤ 18 years	24	9(37.5)	15(62.5)	0.910
	≥18 years	67	26(38.8)	41(61.2)	
Glomeruli Number	≤ 25	44	21(47.7)	23(52.3)	0.079
	≥ 25	47	14(29.8)	33(70.3)	

FIGURE IV:
IMMUNOHISTOCHEMICAL SATIN C4D POSITIVE IN MEMBRANOUS GLOMERULONEPHRITIS



Immunohistochemistry utilization to appreciate the C4d immune-related outcome in kidney disease has beneficial interest on clinical grounds. Currently, the importance of C4d has been established in the pathology and diagnosis of renal biopsies; however, there are fewer publications available. A study in Japan[5] has highlighted the benefits of C4d in Glomerulonephritis compared to Immunofluorescence. Regional data showed focal segmental glomerulosclerosis (FSGS) as the leading histopathological diagnosis among all primary Glomerulonephritis was 29% conducted in Pakistan[10], followed by Membranous Glomerulonephritis seen in 23.5% of cases. In our study, primary focal segmental glomerulosclerosis was 53.8% (49/91), the most common histological diagnosis, followed by Membranous Glomerulonephritis 38.5% (35/91). Our

findings confirm the findings of multiple local studies[11,12] and international studies[13,14]. In all cases of focal segmental glomerulosclerosis, 100% (49/91) were negative for C4d staining. Our findings are comparable with Torbati and Tavakolian[15], who reported similar results as all 14 cases (100%) in their study were negative for C4d.

In the current study, in 91 cases of renal biopsies, 46.1% (42/91) showed positivity for C4d staining in the capillary glomeruli wall. A study by Espinosa et al.[16], reported the frequency of Membranous nephropathy as 52.5% with C4d positivity in 100% of cases, similar to what is observed by the current study showing C4d positivity in 100% of cases of this entity.

A study from the United States of America[17] showed C4d positivity in all 0.2% (34/165) cases of Pre-spike Membranous Glomerulonephritis. In our study, 7.7% (7/91) cases were Pre-spike Membranous Glomerulonephritis, and all showed positivity for C4d staining, similar to the study conducted in the United States of America. Identical results are also reported in a study from Tehran, Iran[15], with 100% positivity of C4d in membranous Glomerulonephritis.

In the current study, cases of mesangiocapillary Glomerulonephritis were 7.7% (7/91), all of which showed positivity for C4d staining in the Capillary Glomeruli wall. A study conducted by Gupta et al. [18] showed a 53.57% frequency of mesangiocapillary Glomerulonephritis, which is very high compared to our research, most likely due to the small sample size. A study by Drachenberg CB et al.[19] reported 22 cases of mesangiocapillary Glomerulonephritis in 519 cases. All cases were positive for C4d, which agrees with the findings of our study. However, study from Iran[15] showed only 02 cases of mesangiocapillary Glomerulonephritis; both were negative for C4d, which could be due to a smaller number of this entity.

CONCLUSION

In conclusion, C4d staining can be used in Membranous Glomerulonephritis, especially in Pre-spike Membranous Glomerulonephritis when the Immunofluorescence of IgG staining is very weak or focal. C4d staining is a well-founded procedure to demonstrate the diagnosis of membranous Glomerulonephritis in kidney biopsy specimens.

The current study showed few hindrances. One of them is the smaller scale sample size, and hence more structured studies with a larger sample are required. This study was conducted in an urban area; therefore, the results might not generalize to larger populations.

Ethical Permission: College of Physicians and Surgeons Pakistan dissertation approval letter No. CPSP/REU/ HSP.2015-201-565, Dated: 23-12-2019.

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AUTHOR CONTRIBUTIONS

Shafique M: Primary investigator
Bukhari U: Manuscript review and final approval
Kumar S: Data interpretation
Raza M: Drafting and writing of the manuscript
Bukhari A: Review & editing

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